

Differential potency and trans-activation of normal and mutant T24 human H-*ras*1 gene promoters

Demetrios A. Spandidos and Alex Pintzas*

The Beatson Institute for Cancer Research, Garscube Estate, Switchback Road, Bearsden, Glasgow G61 1BD, Scotland and

**Hellenic Pasteur Institute, 127 Vas Sofias Ave, Athens 11521, Greece*

Received 17 March 1988

We have employed a short-term transfection assay system in which we monitored the transient expression of the chloramphenicol acetyltransferase (CAT) gene linked to the promoter region of the normal and mutant T24 H-*ras*1 gene or the human ϵ -globin gene in Chinese hamster lung (CHL) cells or cells derived from them which carry and express one or the other of the polyoma virus early genes. Our findings can be summarized as follows: (i) The mutant T24 H-*ras*1 promoter region behaves as a stronger promoter than the H-*ras*1 gene in all these types of cells as well as in rat 208F fibroblast cells. (ii) In CHL cells expressing the polyoma large T antigen the normal and mutant T24 H-*ras*1 promoters are not trans-activated in these cells and only a 2.5-fold activation of the ϵ -globin promoter is observed. (iii) In cells expressing the polyoma middle T antigen both the normal and mutant H-*ras*1 are trans-activated whereas transcription from the ϵ -globin promoter is not affected when compared to the normal CHL cells. (iv) In cells expressing the polyoma small T antigen the normal and mutant H-*ras*1 as well as the ϵ -globin promoters are trans-activated. We suggest from these data that a tissue-specific element exists in the promoter region of the H-*ras*1 gene and that the polyoma middle and small T antigens trigger the expression of proteins that trans-activate these promoters.

Trans-activation; H-*ras* oncogene; Polyoma oncogene

1. INTRODUCTION

The analysis of transcriptional control of the *ras* genes is of special interest, since members of the *ras* family are often activated in human tumors by point mutations [1,2], elevated expression of *ras* RNA transcripts [3,4] and *ras* p21 protein [5,6]. The demonstration that when linked to a strong transcriptional enhancer the H-*ras*1 gene can immortalize [7] or tumorigenically convert [8] cells in vitro suggests that transcriptional control plays an important role in the tumorigenesis induced by the H-*ras*1 gene. Recently, transcription regulatory sequences have been found at both the 5' [9–12] and 3' [13] ends of this gene. Although the *ras* gene is constitutively expressed in many cells it is of in-

terest that some normal tissues express *ras* genes at higher levels [14]. Evidence has been obtained to suggest the presence of a transcriptional enhancer element at the promoter region of the H-*ras*1 gene [10]. Such elements are often found to be trans-activated [15], trans-repressed [16] or to be tissue-specific [17].

There is accumulating evidence to suggest that transcriptional activation by trans-acting mechanisms may be important in oncogenesis [18–20]. A number of viral oncogenes, i.e. adenovirus E1A [21], SV40 T antigen [22] and human T lymphotropic viruses [23], encode proteins with the ability to stimulate transcription. A number of cellular oncogene products share some common properties with these viral gene products, i.e. they are located in the nucleus and it has been suggested that both these types of proteins may regulate transcription of a set of genes important in growth control.

Correspondence address: D.A. Spandidos, The Beatson Institute for Cancer Research, Garscube Estate, Switchback Road, Bearsden, Glasgow G61 1BD, Scotland

Here, we have examined whether expression from the normal and T24 mutant *H-ras1* promoters is affected in cells expressing one or the other of the polyoma virus early genes and have investigated the potency of the two *ras* gene promoters in hamster and rat cells.

2. MATERIALS AND METHODS

2.1. Cells and plasmids

The Chinese hamster lung (CHL) late passage cells and the geneticin-resistant CHL transfectant cell clones FH06LT1-1, FH06MT1-1 and FH06ST1-1 have been described [24]. Clones FH06LT1-1, FH06MT1-1 and FH06ST1-1 were obtained after transfection of early passage CHL cells with recombinant plasmids pH06LT1, pH06MT1 and pH06ST1 carrying the polyoma large, middle and small T antigen gene, respectively. These plasmids also carry the aminoglycoside phosphotransferase *aph* gene which renders recipient cells resistant to geneticin. FH06LT1-1, FH06MT1-1 and FH06ST1-1 cells express the exogenous polyoma genes ([25]; unpublished). The rat fibroblast (208F) cells have previously been described [10].

Plasmids pCAT122A and pCAT123A carry the normal or T24 mutant *H-ras1* gene promoter region linked to the chloramphenicol acetyltransferase (CAT) gene and have been described in [10]. Plasmid pB30 carries the human ϵ -globin gene promoter linked to the CAT gene and has also been described [13].

2.2. Transfection procedure and CAT assay

DNA transfections into recipient cells were performed using a modification [26] of the calcium phosphate technique [27]. Cells were harvested and assayed for CAT activity at 48 h after transfection as in [10,28].

3. RESULTS

The ability of the normal and T24 mutant *H-ras1* promoters as well as the human ϵ -globin promoter to express the CAT gene in CHL cells and in CHL cells expressing one or the other of the polyoma virus early genes was tested by transfecting purified plasmid DNA into these cells and determining the resulting CAT activity 48 h after transfection. The results are shown in fig.1 and tables 1–3. The following points can be drawn from these data. (i) The T24 mutant *H-ras1* promoter behaves as a stronger promoter

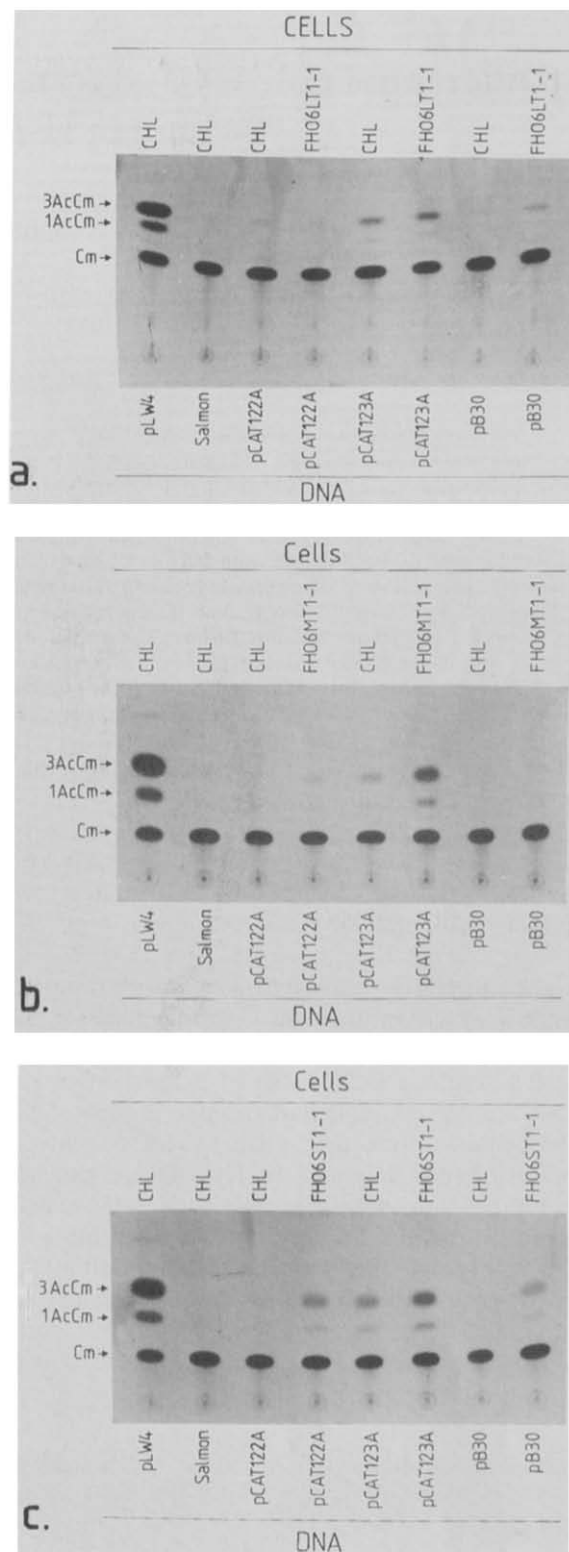


Fig.1. Chromatograms for typical CAT assays upon transfection of CHL cells with CAT recombinants. Experiment using the CHL and FH06LT1-1 (a), FH06MT1-1 (b) and FH06ST1-1 (c) as recipient cells.

Table 1

Relative values of CAT activity in Chinese hamster cells after transfection with CAT recombinant plasmids

Cells ^a	Recombinant polyoma virus plasmid ^b	Exogenous DNA ^c	CAT promoter	CAT activity (average \pm SD) ^d
CHL	—	pLW4	HSV	254 \pm 12
CHL	—	salmon	—	0.7 \pm 0.3
CHL	—	pCAT122A	H-ras1	1.0
FH06LT1-1	pH06LT1	pCAT122A	H-ras1	1.4 \pm 0.3
CHL	—	pCAT123A	T24 H-ras1	8.6 \pm 0.6
FH06LT1-1	pH06LT1	pCAT123A	T24 H-ras1	10 \pm 1.3
CHL	—	pB30	ϵ -globin	1.2 \pm 0.2
FH06LT1-1	pH06KT1	pB30	ϵ -globin	2.5 \pm 0.4

^a CHL, late passage of primary CHL cells; FH06LT1-1, cell clone obtained after transfection of 3rd passage CHL cells with recombinant plasmid pH06LT1, carrying the polyoma large T antigen gene and the *aph* gene for geneticin resistance selection, as in [23]

^{b,c} See section 2 for plasmid derivation

^d Relative values of CAT activity of each plasmid vs that obtained with pCAT122A. The value for CAT activity after transfection of 40 μ g pCAT122A per 5×10^6 Chinese hamster cells was 3.4×10^{-4} pmol acetylated chloramphenicol/ μ g protein per h incubation. Average \pm standard deviation from 3 experiments shown

(8.4–8.6-fold higher) when compared to the normal H-ras1 promoter in these cells. This finding demonstrates a tissue-specific activity, since in our previous study with rat 208F fibroblast cells no significant difference was observed between the promoters [10]. (ii) Whereas the human ϵ -globin promoter is trans-activated by the polyoma large T antigen (from 1.2- to 2.5-fold) neither normal nor mutant H-ras promoters are significantly affected in this respect (fig.1a, table 1). (iii) In CHL cells

expressing the polyoma middle T antigen both the normal and mutant H-ras1 promoters are trans-activated. The CAT values rise from 1.0- to 2.4-fold and from 4.6- to 17-fold, respectively. Transcription from the ϵ -globin promoter is not affected, since CAT values remain almost unchanged (1.2–1.1) (fig.1b and table 2). (iv) In CHL cells expressing the polyoma small T antigen the normal and mutant H-ras1 as well as the ϵ -globin promoters are all trans-activated. The CAT values rise

Table 2

Relative values of CAT activity in Chinese hamster cells after transfection with CAT recombinant plasmids

Cells ^a	Recombinant polyoma virus plasmid ^b	Exogenous DNA ^c	CAT promoter	CAT activity (average \pm SD) ^d
CHL	—	pLW4	HSV	245 \pm 14
CHL	—	salmon	—	0.8 \pm 0.3
CHL	—	pCAT122A	H-ras1	1.0
FH06MT1-1	pH06MT1	pCAT122A	H-ras1	2.4 \pm 0.3
CHL	—	pCAT123A	T24 H-ras1	4.6 \pm 0.4
FH06MT1-1	pH06MT1	pCAT123A	T24 H-ras1	17 \pm 1.5
CHL	—	pB30	ϵ -globin	1.2 \pm 0.2
FH06MT1-1	pH06MT1	pB30	ϵ -globin	1.1 \pm 0.2

^{a-d} See table 1; also FH06MT1-1 carries the polyoma middle T gene

Table 3

Relative values of CAT activity in Chinese hamster cells after transfection with CAT recombinant plasmids

Cells ^a	Recombinant polyoma virus plasmid ^b	Exogenous DNA ^c	CAT promoter	CAT activity (average \pm SD) ^d
CHL	—	pLW4	HSV	253 \pm 14
CHL	—	salmon	—	0.7 \pm 0.3
CHL	—	pCAT122A	H-ras1	1.0
FH06ST1-1	pH06ST1	pCAT122A	H-ras1	8.6 \pm 0.7
CHL	—	pCAT123A	T24 H-ras1	8.4 \pm 0.3
FH06ST1-1	pH06ST1	pCAT123A	T24 H-ras1	20 \pm 1.7
CHL	—	pB30	ϵ -globin	1.1 \pm 0.2
FH06ST1-1	pH06ST1	pB30	ϵ -globin	5.5 \pm 0.4

^{a-d} See table 1; also FH06ST1-1 carries the polyoma small T gene

from 1.0 to 8.6 and from 8.4 to 20 for the normal and T24 Ha-ras1 promoter respectively, and from 1.1 to 5.5 for the ϵ -globin promoter.

In a previous study [10] we found no essential difference in potency of the normal and T24 H-ras1 promoters by assaying the CAT activities of plasmids pCAT122A and pCAT123A in rat 208F fibroblasts. It was therefore of interest to compare our results on CHL cells simultaneously to those in 208F cells. The precise amount of each exogenous DNA in each transfection experiment was checked

by agarose gel electrophoresis in parallel with standard plasmid DNA. The results from three independent experiments are listed in table 4, a representative chromatogram being depicted in fig.2. Although our present results confirm the presence of promoter activities in the normal and mutant T24 ras 0.8 kb DNA fragments they also show that the mutant T24 when compared to normal H-ras1 promoter is more potent in inducing CAT activity in both CHL (4.2-fold) and 208F (5.0-fold) cells. The latter finding represents a discrepancy between these results and our previous data [10] and is not understood at present.

Table 4

Relative values of CAT activity in CHL and rat (208F) cells after transfection with CAT recombinant plasmids

Cell line ^a	Exogenous DNA ^b	CAT promoter	CAT activity (average \pm SD) ^c
208F	pLW4	HSV	69 \pm 3.0
CHL	pLW4	HSV	56 \pm 2.8
208F	salmon	—	0.4 \pm 0.1
CHL	salmon	—	0.2 \pm 0.1
208F	pCAT122A	H-ras1	1.2 \pm 0.1
CHL	pCAT122A	H-ras1	1.0
208F	pCAT123A	T24 H-ras1	5.0 \pm 0.4
CHL	pCAT123A	T24 H-ras1	4.2 \pm 0.5

^a See table 1

^b See section 2

^c Relative values of CAT activity of each plasmid compared to that obtained with pCAT122A in CHL cells. The value for CAT activity after transfection of 40 μ g pCAT122A per 5×10^6 Chinese hamster cells was 8.3×10^{-4} pmol acetylated/ μ g protein per h incubation. Average \pm standard deviation from 3 experiments shown

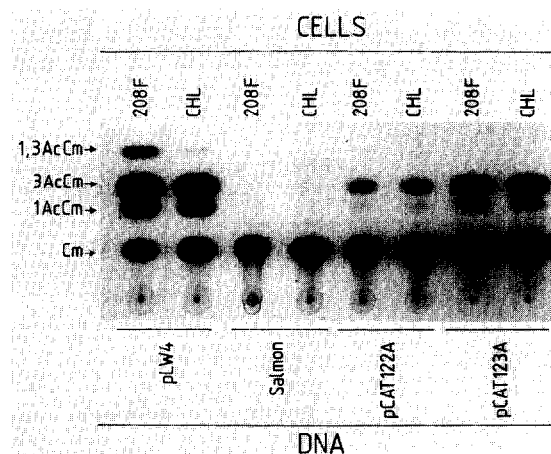


Fig.2. Chromatograms for typical CAT assays upon transfection of Chinese hamster lung (CHL) in rat fibroblast (208F) cells with CAT recombinants.

4. DISCUSSION

We have employed expression vectors in which transcription of the CAT gene is under the control of the human normal or T24 mutant *H-ras1* gene or the human ϵ -globin gene. We have transfected these plasmids into cells which express the polyoma virus large, middle or small T antigen genes. Our transient assay experiments show that the middle and small T antigen genes exert a stimulatory effect on the two *H-ras1* promoters and the large T and small T antigen gene on the ϵ -globin promoter. The question which arises concerns the mechanism of middle and small T antigen action on the *ras* promoters. The proteins could act directly, by binding to DNA sequences, to facilitate specific transcription initiation. Alternatively, the proteins might act indirectly by modifying some component of the transcriptional machinery and thereby allow increased transcription from the *H-ras* promoters. There is as yet no direct evidence to choose between these two possibilities. It would also be of interest to ascertain whether transcription of the endogenous *H-ras* gene is altered in the polyoma transfectant cell lines.

An unexpected but potentially important finding in our studies was the demonstration that the mutant T24 *H-ras1* promoter/enhancer region is more active than the corresponding 0.8 kb *Sst*I DNA fragment of the normal *H-ras1* gene. A possible structural basis for this tissue-specific difference between normal and mutant *H-ras* promoters is a 6 bp deletion in the mutant T24 promoter relative to the normal *H-ras1* gene which is shown in fig.3. It is of interest that this sequence is one of several 5' *H-ras1* sites homologous to the consensus sequence for binding of the Sp1 transcription factor

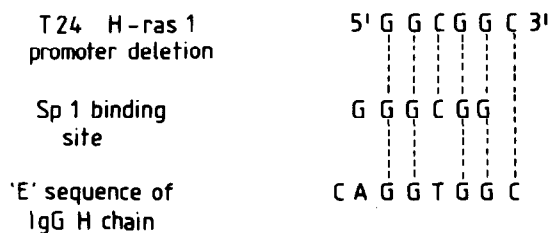


Fig.3. Comparison of the sequence deleted from the promoter of the T24 *H-ras1* gene, the Sp1-binding sequence and the 'E' tissue-specific sequence of the IgG H chain gene.

[29,30]. Some resemblance is also seen to the tissue-specific element 'E' present in the IgG H chain enhancer [31,32].

Further studies should address the importance of these transcriptional effects for the role of *ras* gene expression in initiation and progression of malignant transformation.

Acknowledgements: We would like to thank Dr James Neil for critical reading of the manuscript. The Beatson Institute is supported by the Cancer Research Campaign of Great Britain and the Hellenic Pasteur Institute from the Greek Secretariat of Research and Technology.

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